

# Innovations

## The Mammary Gland: Protein Factory of the Future

The lack of specific proteins can cause a wide range of diseases including diabetes, phenylketonuria, and cystic fibrosis. For the last two decades, scientists have been searching for ways to produce proteins that have therapeutic value using biological reactors. The unique secretory properties of the mammary gland combined with the ability to generate large, transgenic farm animals such as cows, sheep, and pigs, provide scientists with innovative approaches to produce large quantities of proteins in milk. In addition to supplying proteins for therapeutic uses, this technology has significant applications in environmental and toxicological research. Bioengineering of the mammary gland may be useful for investigating potential effects of altered human lactation due to maternal exposure to environmental xenobiotics. The characterization of milk protein genes has also fostered investigations of the regulation of oncogene expression and the induction of cell proliferation by growth factors in mammary tissue. As a consequence, the mammary gland of experimental animals has been genetically engineered to explore the molecular mechanisms involved in the development of mammary tumors.

### A Specialized Secretory Gland

In evolutionary biology, lactogenesis, or milk secretion, is considered one of the most important functions for the survival of mammals. Milk consumption by the new-

born is crucial during the early stages of life. Colostrum, which is produced by the mammary gland postpartum, provides the newborn with immunity and precious nutrients that enhance survival. Normal development of the mammary gland is essential to producing sufficient milk to satisfy the nutritional needs of the offspring, thus ensuring the propagation of the species.

The mammary gland is a specialized gland that synthesizes and secretes milk components into the lumen (cavities) of ducts. The ability to culture mammary epithelial cells in the laboratory on components of breast connective tissue in a petri dish was a major step toward understanding the cellular and molecular events that cause mammary epithelial cells to differentiate. Milk is secreted into small sacs known as alveoli, which are embedded in the connective tissue of mammary glands. Alveolarlike structures, generally referred to as mammospheres, can be generated by culturing mammary epithelial cells.

Advances in molecular biology in the last decade have provided biologists with a better understanding of the developmental, hormonal, cellular, and molecular mechanisms that regulate the expression of milk protein genes, namely, the caseins and whey proteins. Approximately 90% of the protein in the milk of dairy cows is accounted for by four caseins and two whey proteins. Expression of milk protein genes is mediated through activation of

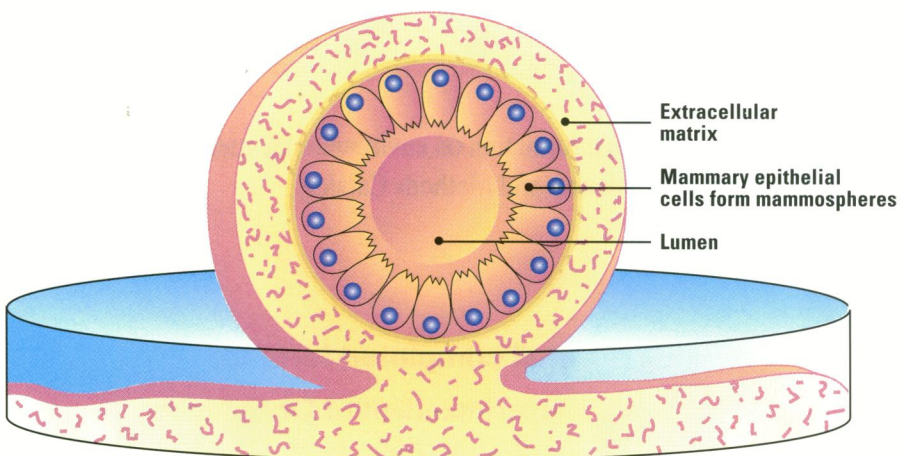
hormone-responsive elements (HRE) in the promoter regions of a gene's DNA. A variety of promoter elements have been characterized that direct the expression of milk protein genes. Two of the casein genes, namely,  $\alpha$ -casein and  $\beta$ -casein, have most likely evolved from a common ancestor gene, and the promoter region of both genes contains HRE sequences that are highly conserved. This evolutionary conservation of the HRE elements is of great significance to mammary gland biotechnologists. The characterization of "universal" transcription regulatory sequences ("transcription modules") is an important prerequisite to producing proteins in the milk of different dairy species.

### Bioengineering

To use the mammary gland as a bioreactor, the transcription modules that drive the expression of proteins must meet two criteria. First, the transcription modules must be mammary specific, and second, expression of the transgene must be under the same developmental and hormonal controls that coordinate the expression of milk protein genes. For example, the onset of lactation is marked by developmental and hormonal events that shift the mammary gland from a pregnant to a lactating state. Mammary epithelial cells differentiate to form fully developed lobulo-alveolar structures, and after birth of the offspring, an increase in circulating lactogenic hormones, specifically glucocorticoid and prolactin, induces the expression of milk protein genes.

The expression of foreign proteins in milk of transgenic animals has most often been accomplished by cloning DNA encoding for the protein of interest downstream of DNA fragments that contain defined transcriptional modules. However, this strategy has not always produced large quantities of proteins because essential regulatory sequences are lacking in the DNA of the transgene. Including additional DNA domains and untranslated regions and using genomic clones or minigenes have resulted in enhanced and proper hormonal regulation of transgene expression.

Sophisticated strategies have recently been designed to confer appropriate developmental and hormonal regulation of transgene expression to the mammary gland. In particular, DNA sequences have been identified that provide dominant regulation of gene expression, such as locus control regions (LCR) and matrix attachment regions (MAR). The LCR and MAR elements may be useful in preventing loss



Joseph Tart

**Mammary magic.** Mammary epithelial cells secrete milk containing desired proteins into the lumen of the mammosphere.

of expression if a transgene integrates in a transcriptionally "silent" region of the chromosomal DNA and in buffering the influence of negative or positive regulatory elements in the DNA.

## A Bioreactor

The bioactivity of many proteins requires post-translational modifications such as glycosylation and carboxylation. These complex modifications may not occur in bacterial systems in such a way as to yield proteins of desired potency. As a result, proteins produced in bacteria may have to be chemically manipulated before recombinant proteins can be used for therapeutic or industrial purposes. Using this technique, mammary epithelial cells have been engineered to secrete proteins of therapeutic interest such as human protein C and tissue plasminogen activator, important mediators of hemostasis and coagulation, and human  $\alpha_1$ -antitrypsin, which is involved in emphysema.






## Lactation and Environmental Health Research

Using the mammary gland to produce proteins may have important implications in environmental health and neonatal toxicology research. Transgenic mammary epithelial cells could provide gene knock-out models to investigate modulation of expression in response to environmental chemicals. Many environmental agents appear to influence the physiology of the lactating mammary gland. For instance, tumor promoters such as phorbol esters have deleterious effects on synthesis and secretion of milk components, in particular the caseins and the whey protein  $\alpha$ -lactalbumin. The phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate induces the activity of the protein kinase C, which in turn alters the phosphorylation of cytoskeletal proteins. These changes lead to dissociation of protein complexes necessary for maintaining cell-cell contacts and induce disruption of cell morphology. As a result, normal cells may take on characteristics of tumor cells. Other compounds such as the potent dioxin TCDD may compromise lipid synthesis and the secretory activity of the mammary gland.

## Potential Pitfalls

Because farm species are an integral component of the natural ecologic system and human microenvironment, the manipulation of the genetic makeup of the mammary gland may be a concern. Using the mammary gland of animals to manufacture proteins may affect the health of the animals. For example, secretion of tissue plasminogen activator in goats' milk and whey acidic protein in pigs' milk was associated

## Therapeutic and Experimental Applications

Species	Transgene	Research Area
	human tissue plasminogen activator	involved in regulation of hemostasis and coagulation
	human growth hormone	potential paracrine effects on mammary growth
	human $\alpha_1$ -antitrypsin	regulates proteolytic degradation of alveolar walls of the lungs, involved in lung emphysema
	hTGF $\alpha$ Ha-ras	growth factor, mammary neoplasia oncogene, breast cancer
	interleukin-2	stimulates T-cell proliferation
	human protein c	involved in regulation of hemostasis and coagulation
	human factor IX	zymogen of serine protease (FIXa) involved in blood coagulation cascade
	insulinlike growth factor-I	growth factor, cell proliferation

## Environmental Research Issues

Environmental Agent	Research Area
phorbol esters	tumor induction, milk synthesis, metabolism of cytokeratins, cell morphology, phosphorylation cascades
dioxins	epidermal growth factor receptor, lipid metabolism, phosphorylation state, immune system
uridine derivatives	lactose synthesis
oxidizing agents	differentiated functions
insecticides	neonatal toxicology
estrogenlike	mammary development, lactation, breast cancer

(Source: F.W. Kari, 1992 Workshop on the Effects on Environmental Chemicals on Lactation and the Nursing Neonate, Executive Summary, National Institute of Environmental Health Sciences.)

with lactational shutdown immediately after birth of offspring.  $\alpha_1$ -Antitrypsin was expressed in the salivary glands of transgenic mice when an  $\alpha_1$ -antitrypsin minigene was under the control of a promoter region of the ovine  $\beta$ -lactoglobulin gene.

It is also important to question whether treatment of human subjects with recombinant milk-derived proteins may cause undesirable side effects. Because recombinant proteins produced by mammary epi-

thelial cells may have biological properties other than the therapeutic one, potential secondary effects on human health should be carefully monitored. Health considerations associated with direct human consumption of milk produced by transgenic animals, however, appear to be of moderate concern because milk produced by transgenic animals would be processed to extract and purify the precious therapeutic proteins.

In addition, converting the mammary gland into a bioreactor must be economically advantageous. In this respect, the relatively inefficient *E. coli* and mammalian cell culture models may soon be replaced by viral and yeast systems that may render bioengineering of the mammary gland economically inefficient. For instance, the yeast *Pichia pastoris* has been used successfully for high-level expression of recombinant proteins such as tumor necrosis factor. Because *Pichia pastoris* is a eukaryote, it can perform post-translational modifications similar to those carried out by mammary cells.

The advantages of bioengineering the mammary gland of farm animals are obvious where the availability of large quantities of natural proteins is a limiting factor. Milk-derived proteins of potential therapeutic and nutritive value could be used for the treatment of chronic and hereditary diseases. Moreover, the use of the mammary gland as a bioreactor may provide a model to investigate the role of peptides involved in modulating the effects of environmental xenobiotics in human lactation and neonatal toxicity.

**Donato Romagnolo**  
**Richard P. DiAugustine**

Donato Romagnolo is a visiting fellow and Richard P. DiAugustine is head of the Hormones and Cancer Workgroup in the Laboratory of Biochemical Risk Analysis of NIEHS.

## SUGGESTED READING

Archibald AL, McClenaghan M, Hornsey V, Simons JP, Clark AJ. High-level expression of biologically active human  $\alpha_1$ -antitrypsin in the milk of transgenic mice. *Proc Natl Acad Sci USA* 87:5178-5182 (1990).

Clark AJ. Prospects for the genetic engineering of milk. *J Cell Biochem* 49: 121-127 (1992).

Hennighausen L. The prospects for domesticating milk protein genes. *J Cell Biochem* 49:325-332 (1992).

Romagnolo D, Akers RM, Wong EA, Boyle PL, McFadden TB, Byatt JC, Turner JD. Lactogenic hormones and extracellular matrix regulate expression of IGF-1 linked to MMTV-LTR in mammary epithelial cells. *Mol Cell Endocrinol* 96:147-157 (1993).

Strijker, Platenburg G, Nuyens J, Pieper F, Krimpenfort P, Eyestone W, Rademakers A, Kootwijk E, Kooiman P, van Veen H, de Boer H. Expression of human lactoferrin in milk of transgenic animals. In: *Harnessing biotechnology for the 21st century* (Ladisch MR, Bose A, eds). Washington, DC: American Chemical Society, 1992; 38-41.

Velander WH, Johnson JL, Page RL, Russel CG, Subramanian A, Wilkins TD, Gwazdauskas FC, Pittius C, Drohan WN. High-level expression of a heterologous protein in the milk of transgenic swine using the cDNA encoding human protein C. *Proc Natl Acad Sci USA* 89:12003-12007 (1992).

Wall RJ, Hawk HW, Nel N. Making transgenic livestock: genetic engineering on a large scale. *J Cell Biochem* 49:113-120 (1992).

# FREE CATALOG OF GOVERNMENT BOOKS

The U.S. Government Printing Office has a free catalog of new and popular books sold by the Government. Books about agriculture, energy, children, space, health, history, business, vacations, and much more. Find out what Government books are

all about. Send for your *free catalog*.

**Free Catalog**

P.O. Box 37000  
Washington, DC 20013-7000

